Epizootiology of *Erynia phytonomi* (Zygomycetes: Entomophthorales) and *Beauveria bassiana* (Deuteromycetes: Moniliales) Parasitizing the Egyptian Alfalfa Weevil (Coleoptera: Curculionidae) in Southern California

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ABSTRACT The incidence of *Erynia phytonomi* (Arthur) and *Beauveria bassiana* (Balsamo) in southern-California populations of the Egyptian alfalfa weevil, *Hypera brunneipennis* (Boheman) (Coleoptera: Curculionidae) was determined during 1979 and 1980. *E. phytonomi* infection normally occurred as a single epizootic in March or April after late-winter rains, and was typified by the results in the 1979 survey. Although incidence was relatively high, the epizootic occurred too late to prevent damage to the crop by feeding larvae. Abnormally heavy rainfall early in the year, such as occurred in 1980, initiated an earlier, longer epizootic, and seemed correlated with lower larval densities. *E. phytonomi* survived temporary dry periods occurring during the epizootic in dried host larvae, called resting larvae, on which the fungus sporulated when suitable conditions resumed. *B. bassiana* incidence was less affected by rainfall. Harvesting of the field, resulting in exposure of surviving larvae to soil-borne inoculum, increased the incidence of *B. bassiana*. Complete harvesting of the field seemed to have an immediate adverse effect on incidence of *E. phytonomi*, but levels of the fungus later reached new peaks.

THE MOST IMPORTANT pest of California alfalfa is the Egyptian alfalfa weevil (EAW), *Hypera brunneipennis*. Like other members of the genus *Hypera*, the EAW is attacked by several fungal pathogens. The most common of these, *Erynia phytonomi*, has long been credited with the almost complete control of the clover leaf weevil, *H. punctata* (Arthur 1886, Herrick and Hadley 1922) and more recently has been discovered to be an important control agent in alfalfa weevil, *Hypera postica*, populations (Harcourt et al. 1974, 1977, Puttler et al. 1980). *E. phytonomi* was recovered from *H. brunneipennis* larvae in 1954, shortly after the weevil's introduction into southern California, and has since been found in yearly epizootics throughout most of the area (Hall and Arakawa, unpublished data). Another common pathogen of *Hypera* species is *Beauveria bassiana* (Rockwood 1916, Hedlund and Pass 1968, Thomas and Poinar 1973). In this study, a comparison of these two pathogens and their effect on *H. brunneipennis* populations in southern California during 1979 and 1980 was made.

Materials and Methods

During this investigation, larval populations of the EAW present in untreated alfalfa fields in the San Pasqual Valley of San Diego County, Calif., were sampled. In 1979, the study field was not completely harvested until after sampling ended, although a few small strips of the field were cut during the sampling period. During the 1980 survey, studies were initiated in a field designated as field A. In midseason this field was completely harvested, making sampling impractical until some regrowth of the foliage occurred. Thus, sampling was started in a second field, designated as field B, which was on the same farm and ca. 0.5 mi (ca. 0.8 km) from field A. As an *E. phytonomi* epizootic was also in progress in this second field, sampling was continued in both fields throughout the rest of the study.

For each sample, larvae were collected either by picking six alfalfa stems from a randomly selected 0.09-m^2 quadrant, or by taking 180° sweeps with a standard sweep-net. Stem samples and debris from sweep-nets were taken to the laboratory in plastic bags for separation and examination of all EAW larvae collected.

For the first three sampling dates of the 1980 season in field A stem samples only were taken as younger larvae predominated, making sweeping impractical. On the next sampling date, both stem and sweep samples were taken. The percent infection levels obtained from these two samples (8.9 and 6.6%, for sweep and stem samples, respectively) were approximately the same. Since stem samples required much more time for processing, they were terminated at this point and only sweep samples were taken. The first sample taken from field B consisted of both stem and sweep samples. When

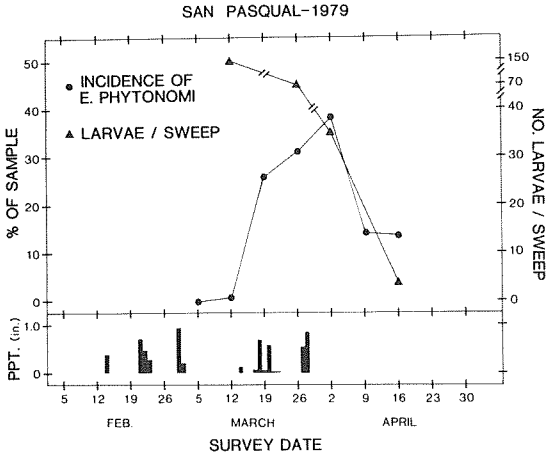

Fig. 1. Seasonal incidence of *E. phytonomi* in larval populations of *H. brunneipennis* from an alfalfa field in the San Pasqual Valley in 1979.

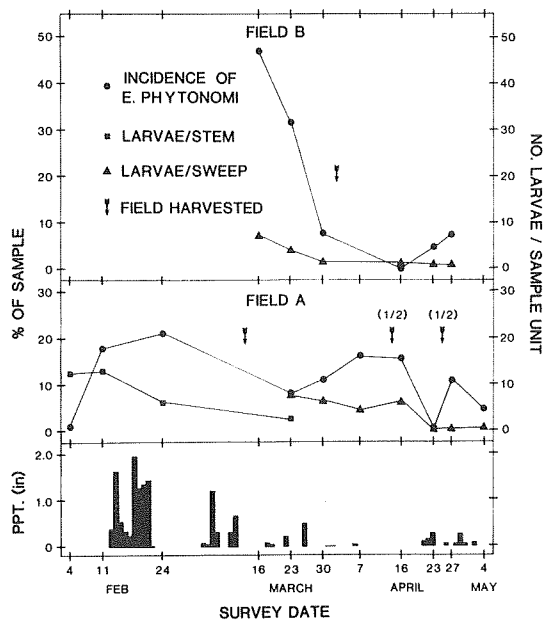

Fig. 2. Seasonal incidence of *E. phytonomi* in larval populations of *H. brunneipennis* in two separate alfalfa fields in the San Pasqual Valley in 1980.

the levels of *E. phytonomi* infection in both sweep and stem samples were found to be close (48.4 and 37.9%, respectively), subsequent sampling was done by sweep-net.

Incidence of disease was determined either by rearing collected larvae or by dissection. Larvae were at first reared individually in small plastic vials in which a small alfalfa spring was anchored in moist sterile sand. Later, groups of 10 to 20 larvae were reared in 90-ml ice cream cups lined with moist, hardened plaster of Paris (modified from Cothran and Gyrisco [1966]). Sprigs of alfalfa were provided for food. Larvae were examined daily for 1 week, with all dead larvae removed and the cause of death determined. The humidity conditions provided in the containers were sufficient to support sporulation of both *E. phytonomi* and *B. bassiana*, making determinations easy.

Larvae to be dissected were killed and stored in 70% EtOH. Dissections were made in acetocarmine on microscope slides. Squashes of the dissected larvae were examined under a microscope for any hyphae or hyphal bodies. The acetocarmine helped to delineate hyphae (Hall and Bell 1963).

Rainfall measurements for the study area were obtained from the U.S. Environmental Data Service, Climatological Data for California.

Results

During the 1979 survey, the *E. phytonomi* epizootic did not begin until after three moderate to heavy rains, at which time larval numbers had reached levels of 100 to 200 larvae per sweep (Fig. 1). No *E. phytonomi*-infected larvae were recovered until 12 March. Infection levels rapidly increased to a peak of 38.3%, then dropped just as rapidly to ca. 14%. As the epizootic progressed, numbers of living larvae decreased rapidly, with the result that no samples were taken after 16 April, because larval numbers were too low to make additional collection practical.

Incidence of *B. bassiana* was very low during the 1979 survey. In only two samples were any *B. bassiana*-infected larvae recovered, and incidence levels never exceeded 1% of the sample.

In 1980, the first incidence of *E. phytonomi* in field A was observed in the 4 February sample (Fig. 2), a full month earlier than that found the previous year. The peak incidence in field B (46.8%) was obtained from the first sample (16 March), indicating that the epizootic in this field was in progress and probably was simultaneous with that in field A. The peak incidence in field A (20.1%) was less than half that of field B. In both fields, however, the fungus reached peak activity before much damage was done to the crop by the larger 3rd- and 4th-instar larvae. Weevil populations in either field never exceeded the economic threshold level of 20 larvae per sweep (Anonymous 1981).

Occasionally, during dry periods between rains, a form of the fungus different than the usual conidial (Fig. 3a) or resting spore stages (Fig. 3d) was found in the field. EAW larvae were obtained with no noticeable external fungal growth other than rhizoids which firmly attached the larvae to the foliage. These larvae were dry, slightly shriveled, very hard, and reddish-brown in color (Fig. 3c). For want of a better term, these dried larvae have been referred to as "resting larvae." When brought to the laboratory and placed under favor-

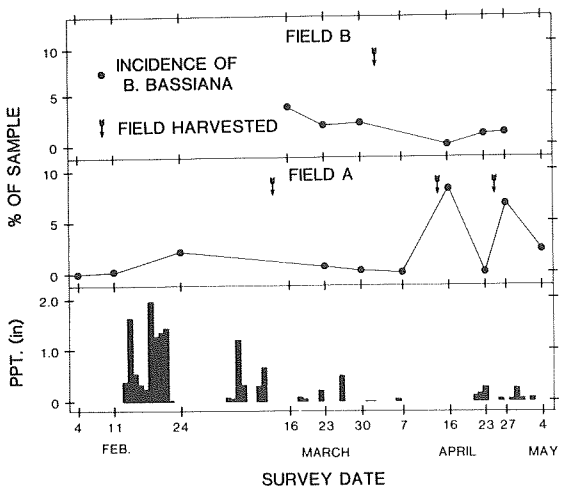

Fig. 4. Seasonal incidence of *B. bassiana* in larval populations of *H. brunneipennis* in two separate fields in the San Pasqual Valley in 1980.

doubt responsible for triggering this early epizootic, probably by washing down the younger larvae to the soil surface, where disease inoculum in the form of resting spores must occur. In a year of normal rainfall, lighter rains may not knock enough of the younger larvae to the soil and the epizootic would not be initiated until more of the older, more exposed, and more easily dislodged larvae were present in the population. Once the epizootic was initiated from inoculum in the soil, subsequent inoculum in the form of conidia would be produced directly in the alfalfa canopy from infected larvae attached by rhizoids to the foliage. Only occasional light rains may then be needed to provide the necessary humidity conditions for sporulation and infection. The resting larvae observed in the field may serve to carry the fungus through temporary dry periods, providing immediate inoculum sources when conditions become more favorable.

The occurrence of the *E. phytonomi* epizootic early in the 1980 season appears to be correlated with the lowered larval numbers in both sampled fields. Whereas mortality directly due to the rain must be considered as a contributing factor, the early and sustained fungus-induced mortality was probably a major reason for the subeconomic levels.

The difference in incidence of *E. phytonomi* in the two fields sampled during 1980 was probably due to the early harvest of field A. Harvesting would have an immediate, adverse effect on incidence by removing large numbers of susceptible hosts and sporulating cadavers attached to the foliage. But harvesting would also serve to expose most of the surviving larvae to the soil with its fungal inoculum, initiating some additional infection and allowing incidence levels to reach a new peak. Incidence levels in field A did, indeed, have a second peak on 7 March, which was considerably higher than concurrent levels in field B. The harvesting of field B apparently had less effect on *E. phytonomi* incidence occurring in that field, since the cutting was done when infection levels had already declined. This decline probably was due to a period of dry weather, since infection levels rose in field B when additional rain fell, as they did in field A.

In contrast to *E. phytonomi*, *B. bassiana* incidence was affected to a lesser degree by rainfall, with the highest incidence of *B. bassiana* occurring during a dry period. This peak incidence was correlated with harvesting of the field, and the resulting exposure of susceptible larvae to soil-borne *B. bassiana* inoculum. Heavy rain may also drive larvae down to the soil. The slight correlation of *B. bassiana* with rainfall, therefore, may not be due to enhanced moisture conditions, but to the disturbance of the foliage-inhabiting larvae.

Larval mortality due to *B. bassiana* was erratic, never occurring in large-scale epizootics such as *E. phytonomi*. This is undoubtedly due, in part, to the difference in inoculum dispersal. Larvae infected with *B. bassiana* are not attached to the foliage as are *E. phytonomi*-infected weevils, and they generally fall to the ground. The chance of *B. bassiana* inoculum produced at the soil level reaching larvae in the alfalfa canopy is far less than that of *E. phytonomi* conidia ejected from cadavers attached to foliage in the canopy itself.

As yet, there is no experimental evidence to support the assumption that resting spores occurring in the soil form an inoculum reservoir for *E. phytonomi*. Laboratory-reared larvae exposed to moist soil from alfalfa fields never yielded *E. phytonomi* infections. Such exposed larvae did, however, consistently develop *B. bassiana* infections, with up to 37% of the test larvae exposed to soil from field A becoming infected (Johnson, unpublished data). Since the conditions initiating germination of and infection by resting spores are still poorly understood, it is possible that more exact conditions are necessary to obtain infection by *E. phytonomi* in this manner.

The results of this study indicate that peak incidence levels of *E. phytonomi* in EAW populations are considerably lower than those found in *H. postica* populations by other workers. Harcourt et al. (1974) found larval mortality due to *E. phytonomi* to range from 65 to 95%, and Puttler et al. (1980) reported incidence levels exceeding 90%. Although the occurrence of *E. phytonomi* in *H. postica* populations is believed to be a recent association (Harcourt et al. 1974), the fungus has been observed in *H. brunneipennis* populations in southern California for nearly 30 years (Hall and Arakawa, unpublished data). Harcourt et al. (1977) felt that *E. phytonomi* was density dependent but overcompensating, and that *H. postica* populations were tending toward stability after the initial disruption by the fungus. Based on this interpre-

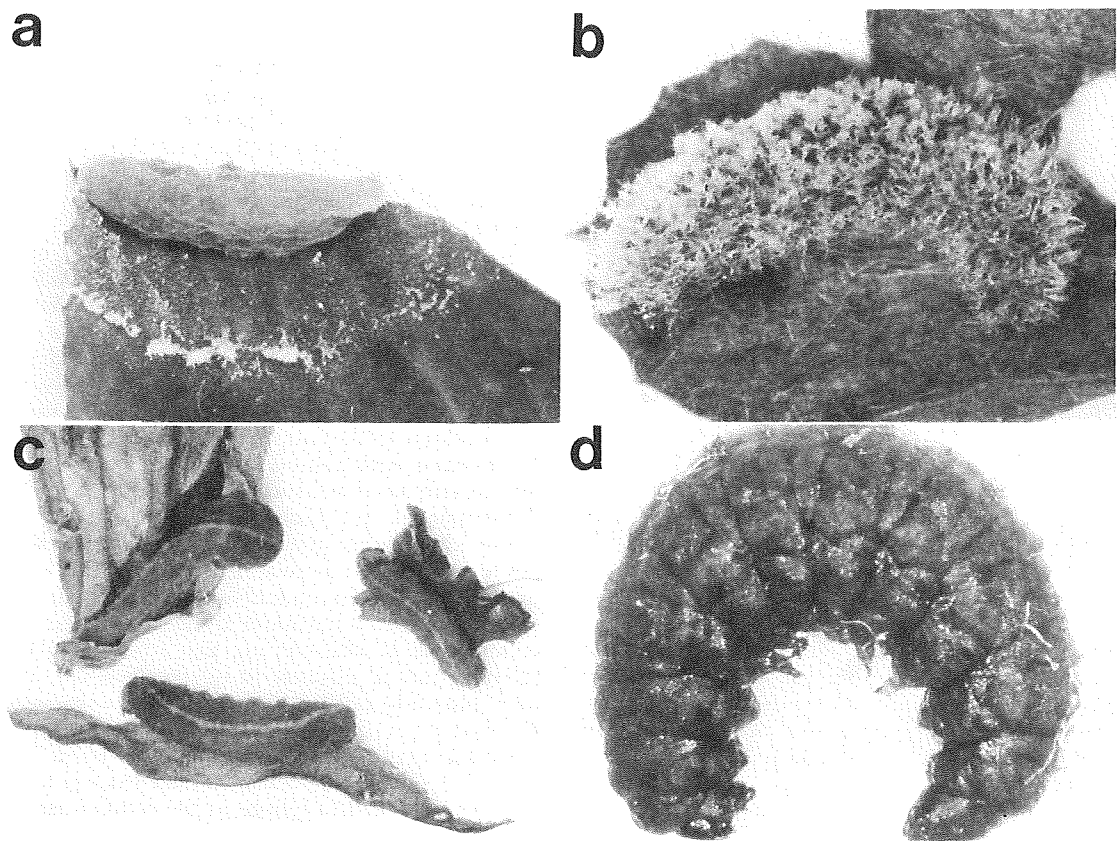

Fig. 3. *E. phytonomi* infecting larvae of *H. brunneipennis*. (a) Conidial stage of *E. phytonomi*. (b) Abnormal vegetative growth of *E. phytonomi*, typified by long, twisted, sterile hyphae, apparently a result of excessive humidity. (c) "Resting larvae" of *E. phytonomi*. (d) Resting spore stage of *E. phytonomi*.

able humidity conditions (relative humidity of ≥95%), even after storage at 5°C for up to 27 days, the fungus in each larva would resume development and sporulate normally to produce conidia. *E. phytonomi*-infected larvae held in the laboratory at relative humidities of ≤80% also dried up and were identical to those dried larvae collected in the field.

B. bassiana was more prevalent in larval populations during the 1980 season (Fig. 4), although levels of activity remained low. The highest incidence (8.4%) was obtained in field A on the 16 April sample date. This peak occurred during relatively dry weather, shortly after half of the sampled field was harvested. Samples taken on the same day from field B yielded no *B. bassiana*-infected larvae. The 23 April sample from field A showed no *B. bassiana* incidence, but the next sample, taken after the second half of field A was harvested, again showed a relatively high incidence. A similar phenomenon was not observed after the harvest of field B, since the entire field was cut and samples could not immediately be taken.

Discussion

It is axiomatic that fungal epizootics in insect populations are nearly always correlated with high moisture conditions, most often in the form of rainfall. *E. phytonomi* epizootics are no exception. In southern California, the epizootics most commonly follow the pattern illustrated in Fig. 1, occurring in a single peak, usually in March or April, shortly after late-winter rains (Hall and Arakawa, unpublished data). The total mortality due to the fungus in the course of the epizootic may be considerable, as in 1979. The rapid drop in the larval numbers shown in Fig. 1, however, was due to the onset of pupation rather than fungus-induced mortality, which occurred too late to prevent damage to the crop by the abundant feeding larvae.

The 1980 epizootic differed from the usual pattern, beginning a month earlier when the host population consisted mostly of younger larvae. Abnormally heavy rains (total precipitation for the months of January, February, and March in 1980 was 27.06 in. [ca. 69 cm], compared with 13.72 in. [ca. 35 cm] for the same months in 1979) were no

tation, it may be construed that stability has been reached in EAW populations, resulting in lower incidence levels. Further, the EAW is adapted to more arid conditions than the alfalfa weevil. Consequently, rainfall patterns in the *H. postica* range may be more suitable for greater activity by *E. phytonomi*. Whatever the reason for the relatively lower incidence levels of *E. phytonomi* in *H. brunneipennis* populations, the fungus remains a major biotic mortality factor for the EAW.

Acknowledgment

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